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Nanoparticulate Drug Delivery in Pregnancy: Placental Passage and Fetal Exposure

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Abstract: During the past decade there has been an explosion in the number of nanoparticulate drugs or drug delivery systems being explored, developed and marketed for the treatment and prevention of human diseases. While the potential dangers of drug administration in pregnancy are well known, there are circumstances where the benefits of maternal drug administration are perceived to outweigh the risks to the fetus. Hence, the administration of potentially harmful drugs in pregnancy is surprisingly common. Nanoparticulate delivery systems offer a potential avenue for delivering therapeutics to maternal tissues with minimal risk of incidental fetal exposure, depending on the ability of the nanoparticle in question to cross the placenta. As the conduit to the fetus, the placenta is both a drug target and a drug barrier, as well as a potential target of any toxicity. Limited studies on this topic show considerable uncertainty regarding the transplacental passage of nanoparticles, and our understanding of the criteria that determine transferability is poor. Despite the fact that the toxicity caused by environmental and man-made nanoparticulates has been widely studied in various organ systems, data on the effects of maternal nanoparticle exposure on human fetal tissues are lacking, although studies in rodents indicate that caution is justified. In this review, we examine the evidence relating to the potential toxicity of nanoparticles in pregnancy, the ability of the placenta to take up and transfer nanoparticles to the fetus, and the theoretical benefits and risks of administration of nanoparticle-based therapeutics in pregnancy.

Keywords: Fetus, nanoparticle, pharmaceutical, placenta, pregnancy, uptake.

NANOPARTICLES: DESCRIPTION, DEFINITION AND HUMAN EXPOSURE

The term nanoparticle (sometimes also referred to nanomaterials, Aitken mode particles, nucleation mode particles or ultrafine particles) is applicable to any particle that has at least one dimension between 1-100 nm, although sizes up to 1000 nm can be included, depending on the context and discipline [1, 2]. In comparison, the smallest living entities**,** such as virus particles**,** typically measure around 20-100 nm in diameter, mycobacterium are 200 nm or more, and most bacteria measure >500 nm. Naturally occurring biological nanoparticles can be composed of polymeric macromolecules such as polypeptides, polysaccharides and glycolipids [3], as well as minerals and crystals. Man-made nanoparticles can be fabricated for industrial, medical or scientific purposes (e.g. semiconductors, catalysts, photovoltaic cells, drug delivery agents). Alternatively, they are also produced unintentionally as a result of industrial processes (e.g. diesel exhaust), or erosion (e.g. the wear and tear of metallic prosthetics), and are associated with environmental pollutants and toxicants.

It is predicted that nanosized materials will have significant impact on almost all industries and all areas of society within the foreseeable future as their applications become established**,** and issues relating to toxicity and safety become better understood and managed. Currently, while the toxicity and safety of nanoparticles is the subject of active investigation [4], the development of therapeutic applications for nanoparticles is equally an area of intense activity [2, 3, 5, 6]. Applications of nanoparticles in medicine include bioimaging, diagnostics and pharmaceutics; there are now numerous journals, societies and international conferences dedicated to the burgeoning discipline of nanomedicine [7]. With the anticipated forthcoming explosion of nanoparticles in many aspects of human activity, it is inevitable that exposure at all stages of life will become more commonplace.

The particular focus of this review is the clinical application and safety of nanoparticle-based drug delivery in pregnancy; however, insight from other areas and disciplines will be considered where relevant. We will first give a brief overview of the general properties and applications of nanoparticles in medicine, counterbalanced by issues of toxicity and safety. Then, we will survey current knowledge on nanoparticle exposure in pregnancy and the uptake and transfer of nanoparticles by the placenta to fetal tissues. Finally, we conclude with a discussion of the particular applications of nanoparticles to obstetric medicine**,** and the challenges that must be faced in order to bring the promise to fruition.

NANOPARTICLES AS DRUG DELIVERY AGENTS: ADVANTAGES AND KEY PROPERTIES

Due to their unique size, nanoparticles exhibit a very high surface area per unit mass, conferring upon them distinct

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physico-chemical properties such as enhanced reactivity and bioactivity [8]. Indeed, nanoparticles smaller than 50 nm are subject to the laws of quantum mechanics rather than classical physics [9]. The realization that nanoparticles have distinct advantages for therapeutic drug delivery has lead to an explosion of research in this area [3]. In theory, nanoparticles can extend a drug's half-life, lower its therapeutic dose, reduce off-target side effects, and target delivery to specific tissues or organs [3, 10, 11]. However, devising systems that exhibit these qualities remains a considerable challenge for nanopharmaceutical science [3, 12]. Non-targeted delivery systems have been developed and tested *in vivo*, and several have made their way to clinical trials and the market, albeit restricted to limited applications [10, 13]. Typically, a nanoparticle drug delivery system needs to be able to encapsulate the drug cargo, survive destruction or elimination as it circulates through the body, enter the target organ through the endothelium and extracellular matrix, penetrate the desired cell type, evade endosomal or lysosomal destruction, and undergo disassembly to release its contents, all without causing immune activation or cellular or systemic toxicity. Alternatively, under some circumstances, release of the drug cargo into plasma, airways or phagocytic cells could be the desired outcome, depending on the nature of the pharmacological target, while for other applications an inability to penetrate a biological barrier (e.g. the blood-brain barrier or placenta) could be a distinct advantage in terms of preventing unwanted exposure and side-effects. A limitation of most nanoparticulate drug delivery systems is a general restriction to parenteral or topical administration. Orally active formulations are seldom discussed in this context, although there have been some attempts to design and fabricate orally-active nanoparticulates that have had encouraging results [14, 15].

The clinical use of nanoparticle delivery agents depends to a large extent on their biodistribution and pharmacokinetic characteristics [10, 16]. The key variables affecting nanoparticle pharmacokinetics are chemical composition, hydrodynamic diameter, shape, surface charge and pH [10, 17], all of which can be tuned for a particular clinical application [18]. Particle half-life in the circulation principally depends on renal elimination and the ability to evade sequestration by the reticuloendothelial system (RES) [19]. This mononuclear phagocyte system clears a variety of molecules and particulates from the circulation within seconds to minutes *via* immune cell phagocytosis and/or liver and spleen sequestration / elimination [19, 20]. Opsonisation is a key step in this process [21]. A variety of strategies can be employed to reduce opsonisation and prevent phagocytic activation (see below) [19]. For pharmaceutical targets in the bloodstream this is sufficient, but for nanoparticles with intracellular targets there are several more barriers. If clearance *via* the RES is avoided, the next barrier to overcome is the endothelium. Endothelial pores or fenestrae have diameters 40-100 nm, amenable to entry by nanosized particles [10]. Under some circumstances (e.g. in inflamed or tumor tissues), these can expand to allow the entry and accumulation of particles up to 400 nm, the so-called "enhanced permeability and retention" (EPR) effect. Next, the particle must penetrate the extracellular matrix, a dense network of polysaccharides and fibrous proteins that can create considerable resistance to the transport of macromolecules and nanoparticles [22]. Diffusion through this matrix typically occurs with time, although the proportion of particles that reach the target may be attenuated. Nanoparticles can then cross cell membranes *via* some form of endocytosis (receptor-mediated or otherwise), active transport or diffusion. Positively charged nanoparticles with a degree of lipophilicity generally exhibit efficient transfer across the lipid bilayer [23]. The contents of most endocytotic vesicles are destined for destruction *via* lysosomal fusion. Hence, it is crucial that the nanoparticle evade lysosomal destruction to complete its journey. Finally the particle must be biodegradable and able to release its contents over time to allow interaction with its intracellular target – enzymes, receptors, RNA *etc*. This final disassembly would ideally be mediated by exposure to a key aspect of the intracellular milieu.

Nanoparticle size is considered one of the most important characteristics that determine the penetration of a cellular or membranous barrier as it determines whether the particle is subject to transcellular or paracellular diffusion, or endocytic uptake [24]. Most particles being trialed clinically have dimensions in the order of 5-50 nm [10]. Particles 10-100 nm have enhanced diffusion into tissues and reduced clearance *via* the kidneys, liver or spleen. Size also correlates positively with opsonisation. Surface composition determines pH, zeta potential/charge and hydrophilicity, all of which influence interaction with biological surfaces. A positively charged surface facilitates cellular uptake by association with the negatively charged cell membrane, but also increases adsorption of negatively charged plasma proteins in the circulation. This enhances opsonisation**,** leading to phagocytic uptake and elimination, rendering the nanoparticle ineffective [25]. Hydrophilic conjugates can be bound to the surface to reduce this problem as well as increase size. Increasing hydrophilicity can also reduce particle aggregation, an important aspect for minimizing toxicity [26].

Nanoparticle cores can be fabricated from many different materials, including proteins, lipids, polymers and carbon structures. These are used to produce various delivery systems**,** including polymeric nanocapsules, dendrimers, micelles, liposomes, emulsions, nanogels, carbon fullerines and inorganic nanoparticles. While these exhibit a range of pharmacokinetic properties, the characteristics of each can be manipulated further through post-functionalisation [5, 10, 27-29]. Polymers are now commonly used in nanoparticle construction, although lipid-based carriers are still prevalent [30, 31]. Indeed, recent progress has been made in preparing cationic lipidoids that are capable of highly efficient delivery of siRNA *in vivo* and silencing of clinically relevant target genes [32]. Cationic polymers with either linear or branched structures can be efficient nanocarriers due to their ability to condense a large amount of cargo. Examples of polymers used to construct nanoparticle cores include poly(DL-lactide-co-glycolide (PLGA), polylactic acid (PLA), poly-ε-caprolactone (PCL), poly-alkyl-cyano-acrylates (PAC), N-(2-hydroxypropyl) methacrylamide (HPMA), gelatine, dextran, and chitosan [10, 13, 33]. Polyethyleneimine (PEI) is a commonly used polymer employed as a carrier skeleton for both local and systemic applications; it has been used mainly to encapsulate DNA, siRNA and polypeptides [34-37]. Several *in vivo* studies have been successfully carried out with

this polymer; while there have been some toxicity concerns associated with the use of PEI at high doses, strategies to mitigate toxicity have been successfully adopted [25].

A particularly useful characteristic of nanoparticles is that their biodistribution can be readily altered by minor modifications of the surface 'corona'. Polymers such as polyethylene glycol (PEG) are commonly used as functionalisation agents**,** while other surface modifications are incorporated to achieve non-specific endocytosis and endosomal escape. The addition of PLGA, for example, to the surface of nanoparticles has been shown to allow rapid endosomal escape by selective surface charge reversal once in contact with endosomal pH [38]. Recent research into the use of covalently attached polymers has illuminated potential methods to evade the monocyte-macrophage system. Derivatives of PEG are particularly promising in conferring so-called 'stealth' properties [24]. The hydrophilicity and steric repulsion of surface PEG molecules reduces the opsonisation and activation of complement [39]. PEGylated polymeric nanoparticles, therefore, have the ability to evade the phagocytic cells of the liver and spleen and have been described as 'long-circulating' drug delivery systems. Varying PEG length and surface density confers an additional level of control. Almost all variations of PEG chains confer colloidal stability, but the length of the PEG chain can have a remarkable influence on biodistribution and clearance [24]. Dense PEG shielding over a negatively charged surface and smaller nanoparticle diameter further enhance the avoidance of opsonisation [40]. Recent studies in non-human primates have shown that targeted PEGylated cyclodextran cation nanoparticles are able to deliver non-modified siRNA safely and effectively without toxicity and only a modest immune response [41]. Due to their low toxicity and immunogenicity**,** PEG-enhanced polymer nanoparticles have been approved for clinical use [10, 25].

Carbon fullerines ("Buckyballs") have also been investigated as drug delivery agents with considerable success [42-44]. Advantages include good capacity for hydrophobic drugs, strong core structure and ready functionalisation to modify hydrophilicity, size and charge. Modified carbon fullerene structures have recently been reported to be able to efficiently deliver genes and siRNA to target tissue *in vivo*, due in part to their greater stability in serum, endosomal resistance and rigid structure [45].

One of the attractive properties of nanoparticle drug delivery systems is the ability to target the drug to specific tissues. To some extent this is the "Holy Grail" of nanoparticle drug delivery design, although it is a goal that has been difficult to achieve clinically. Recently, however, investigators have incorporated the addition of functional ligands such as aptamers, polysaccharides, peptides, vitamins and immunoglobulins to target specific receptors for entry into selected tissues, with encouraging results [41, 46-52]. Clearly, this is an area where significant progress is being made. Nuclear targeting is also of interest in some applications, although the challenges of accomplishing drug release in the nucleus while evading disassembly in other cellular compartments are considerable [53].

TOXICITY AND SAFETY OF NANOPARTICLES IN PREGNANCY

The last century has seen a dramatic rise in inhaled nanoparticles from anthropogenic sources. In addition to inhalation (lung), the emerging nanotechnology industry will expose humans through the skin, the gastrointestinal tract, and injected nanomaterials [8], raising concerns over safety. Nanotoxicology, the study of the effects of engineered nanomaterials on living systems [54], is a field that has risen in response to these concerns. Most of the data on nanoparticle toxicology has been based on exposure to airborne nanoparticles (termed ultra-fine particles in this context). Upon inhalation**,** these are deposited on respiratory epithelial surfaces and may provoke inflammation and granuloma formation in lung tissue [55]**,** and induce systemic side-effects such as pro-thrombotic states, cardiac edema, and systemic inflammation [55, 56]. Human lungs have highly efficient mechanisms for clearing debris. Despite this, several studies have demonstrated an uptake of nanoparticles into the respiratory epithelial cells and thus into the circulation [57, 58]. Once in the blood stream, there is the potential to be distributed to any tissue, including the developing fetus, to accumulate and cause toxicity. Due to its rapid growth and differentiation, the fetus represents a particularly vulnerable and sensitive target of nanoparticle toxicity [59].

Diesel exhaust (DE) is a complex mixture of particulates, vapor-phase compounds and gases including nitrogen dioxide, carbon monoxide and sulfur dioxide, and includes thousands of soluble organic compounds. The particulate matter consists of organic carbon based particles of variable size, mostly smaller than 2.5 μm [60]. DE is, therefore, a convenient source of potentially toxic nanoparticles for investigating nanotoxicity in pregnancy. A Japanese study has confirmed that exposing pregnant rats to DE disturbs fetal differentiation of the testis, ovary and thymus [61]. More than 90% of the DE particulate matter in this study was smaller than 500 nm in diameter. However, their findings suggested that the gaseous component, rather than the particulate matter, were the primary culprits for the effects on the endocrine system [61]. It was not possible to determine whether these particles crossed the placenta into the fetal circulation or whether they affected placental function enough to affect gene expression in fetal tissues. In a similar study, pregnant mice were exposed to DE constituents [62] for 8 h, 5 days per week from gestational day 2 to 17. The findings of this study suggested that maternally inhaled DE might influence the development of the central dopaminergic system of the CNS and result in behavior disorders. Unfortunately, the actual accumulation of DE particles in fetal tissues was not examined. Recently, Yoshida *et al.* administered DE-derived 14 nm carbon nanoparticles to pregnant mice [63] and reported that *in utero* exposure resulted in a significant acceleration of testicular maturation in male offspring at birth. However, it was not ascertained whether the damage was caused by direct fetal exposure [63]. In other studies, DE-derived nanoparticles have been shown to have effects on fetal reproductive development and spermatogenesis [60, 64], endocrine disruption [65], altered locomotor function [66] and the dopaminergic system [62].

At present it is not possible to accurately quantify the risks associated with exposure to engineered nanomaterials in pregnancy due to the lack of sufficient data in humans, although animal studies suggest there is definite cause for concern. Until this knowledge becomes available**,** many bodies suggest it is prudent to have appropriate precautions and regulations in place to avoid future public health crises [4, 67]. This is particularly relevant for nanoparticles derived from industry or combustion that are likely to gain access to human tissue through environmental or occupational exposure. Such nanoparticles tend to be carbon centered and contaminated with metals [4, 9, 67]. Therapeutic nanoparticles, on the other hand, tend to be biodegradable and contain molecular linkages that can be disassembled intracellularly [68], thus avoiding tissue accumulation and toxicity. Nevertheless, robust safety data would be needed before nanoparticle-based drug delivery systems could be explored clinically in pregnancy.

THE PLACENTA: PERFUSION, PERMEABILITY AND LIMITATIONS OF EXPERIMENTAL MODELS

The placenta plays a key role in determining the extent of fetal exposure to maternally-administered nanoparticles. The placenta acts as a barrier to particulate transfer, but can also be a site of uptake, accumulation and toxicity [69]. It can also be affected in a way that impairs its function and hence impacts indirectly on fetal growth and development. The placenta's structure, cellular composition, functions and blood flow change with gestational age, and hence its ability to act as a barrier or conduit also changes accordingly [69, 70]. Similarly, fetal susceptibility to toxicity also varies during pregnancy according to its stage of development. During the embryonic period**,** the conceptus undergoes rapid growth, cellular differentiation and organ formation. This particularly sensitive period lasts for the first 8 weeks of pregnancy; the remainder of pregnancy (the fetal period) is characterized by ongoing organ growth and maturation.

The human maternal-fetal interface is composed of the placenta and extra-placental membranes which separate the maternal and fetal tissues. The placental barrier consists of the syncytiotrophoblast membrane composed of multinucleated fused cytotrophoblast cells overlying a basement membrane, a discontinuous layer of underlying mononuclear trophoblast cells, some connective tissue containing stromal cells and the occasional macrophage (Hofbauer cells), and finally endothelial cells of the fetal capillaries. Maternal blood, therefore, is in direct contact with fetal chorionic (trophoblast) tissue which is the main barrier separating the maternal circulation from the fetal microvasculature. The fetus is also enclosed in a membraneous sac, consisting of the amnion, chorion and maternal decidua, which is fairly impermeable to most xenobiotic substances in the maternal blood stream [71]. Fetal swallowing provides a route through which substances in the amniotic fluid can enter the fetal circulation. Amniotic fluid can, in theory, accumulate macromolecules that arrive *via* penetration of the fetal membranes or are excreted in fetal urine.

The placenta was originally assumed to be a purely passive organ before the first study showing the active transfer of amino acids was published in 1948 [72]. Since then, studies of human placental physiology and function have revealed that it simultaneously performs the functions of the neonatal kidney, lungs, intestine and liver, and acts as an endocrine organ. The placenta performs exchange functions in two directions; the transport of substances - including all nutrients - from the maternal circulation to the fetus, and the clearance of waste products from fetal to maternal blood [73]. Most of the literature on transplacental transport focuses on the syncytiotrophoblast as the key exchange surface. The polarized syncytiotrophoblast with its microvilli and basement membrane expresses numerous transport proteins, receptors, ion channels, efflux pumps and exchangers [71]. This tissue is structurally complex, unique in terms of its formation and function, and is a major determinant of maternal drug uptake and permeability. In contrast, the fetal endothelium of the microvilli is almost identical to that found elsewhere [74], although placental capillaries appear to have tighter connections with each other than most other continuous capillaries [75]**,** and are perhaps up to two orders of magnitude less permeable than the blood-brain barrier. In other animals that lack a placental lymphatic drainage system, the fetal capillaries provide most of the resistance to diffusion of macromolecules [76, 77]. In the human placenta, this endothelial barrier acts as a restrictive molecular sieve, and is a significant contributor to the overall placental permeability to hydrophilic solutes larger than about 1000 Da [74].

Most of the data on human placental permeability and transfer have been derived from *in vitro* and *ex vivo* studies [78]. Studies on cord blood drug concentrations performed at term show a reasonable correlation between *in vitro* and *in vivo* results, supporting the validity of these models. However, placental transfer is a complex process, and it is likely that *in vitro* models only partially reflect the systems existing between the mother, placenta and fetus *in vivo* [79]. Using such approaches**,** it has been established that the placenta allows ready passage of many lipophilic and amphiphilic drugs up to 500 Da in size, although a sizeable proportion are subject to efflux *via* ABC "drug pumps" [73, 80]. In contrast, compounds of 1000 Da or more exhibit very poor transfer, except in rare instances when specific receptors exist, suggesting that the placenta does not, in general, allow passage of macromolecules such as proteins, DNA and cellular material [81]. A caveat to this generalization is that traumatic damage to the placenta can cause denudation of the syncytium, effectively exposing the fetal capillaries to open access to substances in the maternal circulation**,** and thereby severely impairing the placental barrier. An additional factor of note is that placental permeability is not constant throughout gestation. Around 20 weeks gestation the cytotrophoblast cell layer becomes sparse and attenuated and barely visible upon microscopy. The distance between maternal and fetal blood decreases to only 2-4 microns at term, and may in places be as thin as 1-2 microns [82]. Hence, studies carried out on term placentas may not necessarily be representative of 1st and 2nd trimester tissues, although they are still considered more representative than studies in animals as discussed below [78].

The placenta shows greater species diversity than any other mammalian organs in terms of structure, cellular composition and function [69, 83, 84]. Inter-species differences

have been described in placental permeability, transport activity, blood flow and metabolic activities [85, 86]. In addition, differences in fetal susceptibility to teratogens also exist between species [87], as do rates and extent of fetal development during pregnancy. The animal species that most closely mimic human placental characteristics have a hemochorial histological organization - primarily primates and rodents. Clearly, primates would be the species of choice, but they are expensive and can be difficult to work with, so rats and mice are more commonly used. While the human placenta is hemomonochorial in classification, composed of a single layer of trophoblast (syncytium) in direct contact with the maternal blood, rats and mice have three trophoblast layers (hemotrichorial) in a labyrinthine placenta [86]. This anatomical difference is reflected by different diffusion differences between maternal and fetal circulations, with concomitant differences in permeability to various substances. In addition, in humans the yolk sac is present only very early in gestation, whereas in rodents it exists throughout gestation, encloses the fetus, and performs important transport functions [88]. For example, the transfer of IgG from maternal to fetal circulations in humans occurs *via* Fc receptors in the placenta, which can be readily demonstrated using *ex-vivo* perfusion [89], while in mice the yolk sac is the organ responsible for IgG materno-fetal transport [90, 91]. Importantly, the rodent yolk sac has been shown to be able to endocytose large proteins and nanoparticles (30 nm in diameter) through a fluid-phase pinocytotic mechanism [92]. This could provide a route for fetal transport of synthetic nanoparticles not available to the human placenta. Due to these differences, animal experiments for the study of human placental function and reproductive toxicity should be viewed with caution, although they do have the advantage of allowing experiments to be conducted with an intact materno-fetal compartment [87, 93].

PLACENTAL TRANSPORT MECHANISMS

The mechanisms of transfer across the placenta have been studied and elucidated in some detail [71, 94, 95]. In brief**,** they can be categorised into the following see also Fig. (**1**):

- Passive diffusion
- Facilitated diffusion
- Receptor-mediated uptake
- Endocytosis (including pinocytosis)
- Paracellular entry
- Water-filled transplacental channels

Most pharmacologically active compounds are low molecular weight, poorly-ionised, amphiphilic molecules which cross the human placenta by simple diffusion. This process is fundamentally dependent on the concentration gradient between circulations, does not use energy, and is dependent on surface area, blood flow, the physico-chemical characteristics of the compound**,** and protein binding [78, 96]. Passive diffusion is, therefore, the most common mechanism of substance transfer [73, 97]. Some substances, however, are transported down their respective concentration gradients by a specific membrane carrier protein. This process, called facilitated diffusion, is passive, saturable, uses no energy, and can be in either direction. Such exchangers can employ ion gradients, electrochemical charge and co-exchange mechanisms to facilitate transport. Endogenous compounds such as glucose, nucleosides, amino acids, peptides, hormones and metabolites are the primary substrates for facilitated diffusion [78]. Some substances are taken up into syncytiotrophoblast by facilitated diffusion and then distributed further by passive diffusion alone [71]. The placenta is also a site of considerable active transport, mediated predominantly by members of the ABC family of efflux proteins [73]. Active transport involves the hydrolysis of ATP to drive efflux against a concentration gradient, and is primarily involved with excretion of xenobiotics and potentially toxic metabolites out of the placenta [73, 98]. The majority of active transport in the placenta is in the maternal direction, consistent with a protective, excretive function. However, some ABC transporters face either the placental stroma or the fetal capillary lumen, in which case they pump towards the fetal circulation [73]. Recently it has become apparent that active transport plays an important role in conveying a range of important endogenous compounds across the placenta, as well as helping to protect the placenta from accumulation of potentially toxic metabolites [99]. For a limited number of substrates, receptor-mediated uptake is an alternative mechanism of placental uptake. Examples are immunoglobulins (see below), cobalamin binding proteins [100] and lipid receptors such as the LDL receptors, scavenger receptor A (SR-A), and HDL-binding scavenger receptors B1 (SR-B1) [101]. Such mechanisms are highly restricted to specific targets, however.

The transport processes described above are unlikely to be employed in mediating nanoparticle transport across the placenta**,** mainly due to size restriction (Fig. **1**). The various forms of endocytosis are likely to be the main routes of entry. Endocytosis is an active process involving the invagination of cell membranes to form intracellular vesicles. These can either travel to the opposite polar membrane and release the contents, fuse with lysosomes to destroy the contents, or be recycled to the point of entry [78]. Amongst studies of placental transport mechanisms**,** the most commonly studied protein is IgG. It has been shown that IgG molecules appear to travel across fetal capillary endothelium via a transcellular route involving endocytosis and vesicular transport [102]. IgG (molecular weight 150,000 Da) is readily transported from the maternal to fetal circulation via a receptor-mediated process involving Fc receptors on the apical membrane of the syncytiotrophoblast [95]. It has been proposed that IgG-FcR complex is then internalized by fluid-phase endocytosis, transcytosed and released into the villous interstitium [95]. The mechanism of subsequent transfer across the endothelium is unknown; the fetal endothelial cells are too tightly apposed to allow the paracellular diffusion of such large molecules [103]. However, FcγR receptors have been located on the endothelium of the placental vasculature [104, 105], suggesting that a receptor-mediated diffusion mechanism may function to deliver immunoglobulins from the placenta to the fetal circulation. Interestingly, evidence exists that IgG-bound ligands can also cross the placenta via these mechanisms [95]. For example, the trans-placental transfer of non-human insulin or malarial antigens is dependent on

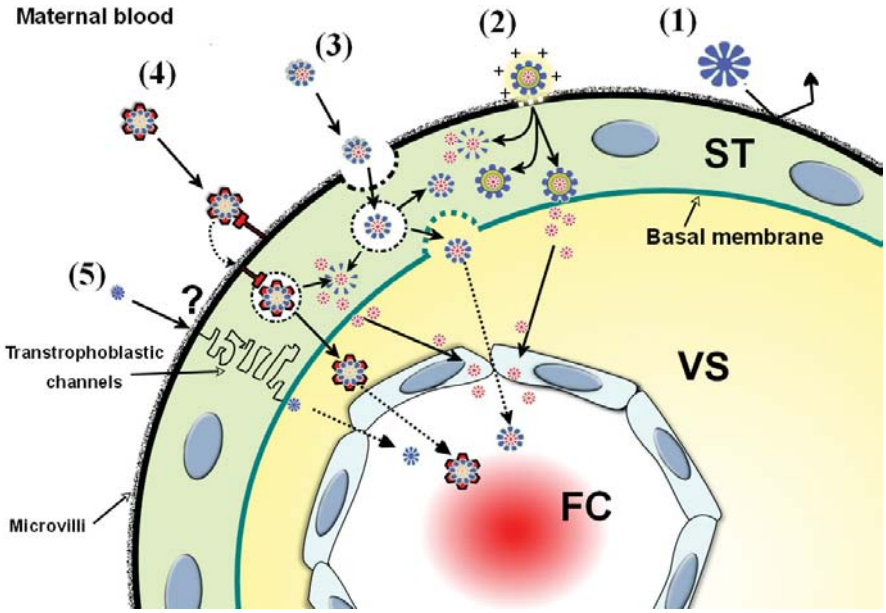

Fig. (1). Uptake and distribution of nanoparticle-based drug delivery agents across the human placenta.
1) Nanoparticles of a certain size, charge or surface composition fail to penetrate the apical membrane of the syncytiotrophoblast (**ST**) and are retained in the maternal circulation. **2**) Cationic nanoparticles (e.g. liposomes) may be able to fuse with the syncytial membrane and enter the cytoplasm, where they can either disassemble and release their contents, remain intact within the cytoplasm, or fuse with the basal membrane and release their contents into the villous stroma (**VS**). Diffusion may then occur through the fetal endothelium into the lumen of the fetal microcapillaries (**FC**). **3**) Nanoparticles may be taken up by endocytosis, and either degraded in endosomes to release their contents in the cytosol, or escape *via* transcytosis into the villous stroma for eventual diffusion into the fetal capillaries. **4**) Ligand-modified nanoparticles can be taken up into the syncytium *via* receptor-mediated mechanism (possibly involving endocytosis) and either degraded, retained or transcytosed, facilitating access to the fetal circulation. **5**) Very small, hydrophilic nanoparticles may penetrate the syncytial membrane *via* putative transtrophoblastic channels and thereby enter the stroma and fetal capillaries. However, the existence of a contiguous network of channels extending through the entire syncytium remains to be proven.

prior binding by specific antibodies in the maternal circulation [81, 89, 106].

The placental syncytiotrophoblast plasma membrane displays many clathrin-coated regions between the microvilli, and coated vesicles are abundant in the underlying cytoplasm [107]. In 1982, Pearce showed that clathrin-coated pits were present in the placenta; when isolated these were shown to contain ferritin, transferrin and IgG [108]. The pits appeared to be in the region of 0.5-1 nm diameter, which is presumably too small for nanoparticle uptake. In contrast, a subsequent study in placental membranes from the macaque found evidence of much larger coated pits and vesicles from 50-500 nm in size [109]. Evidence of a functional endoytotic/pinocytotic pathway able to internalize albumin and hemoglobin has also been presented, confirming the structural/biochemical studies [110, 111] Fig. (**1**). It should be remembered, however, that endocytotic uptake into trophoblasts does not necessarily equate to transport to the fetus (see later).

A solid body of evidence, primarily based on electron microscopy following perfusion with electron-dense lanthanum hydroxide, suggests that there are narrow, winding, highly branched tubular channels extending from invaginations in the basal lamina through the syncytiotrophoblast of the human, rat, rabbit and guinea pig placenta [112, 113]. The purported function of these "transtrophoblastic channels" is to accommodate fetal-to-maternal bulk flow. Under normal conditions their diameter has been calculated to be around 20 nm, capable of transporting substances around 1 nm diameter or less, but under pressure they can dilate to many times this size [114]. Supportive evidence for the existence of such paracellular pathways comes from the observation that transplacental transport of small inert hydrophilic molecules is approximately proportional to their diffusion coefficients in water [115]. However, anatomical evidence that these channels actually extend through the trophoblast and open through the syncytial apical membrane is still lacking, more than 10 years after their original description. Numerous studies suggest that erythropoietin (molecular weight 30400 Da)**,** and even relatively small proteins such as insulin**,** cannot pass through the placenta [96, 116]. These observations strongly argue that a system of 20 nm contiguous water-filled channels cannot exist in the placenta, at least under normal physiological conditions.

PLACENTAL PERMEABILITY TO NANOPARTICLES: THEORY AND EVIDENCE

Data from Animal Studies

There have been several studies published of fetal nanoparticle exposure in pregnancy using experimental animals, most of them concerned with the toxicity of environmental nanopollutants. Fujimoto and colleagues studied the effects of DE on the placenta by measuring the expression of placental cytokines following maternal DE exposure [117].

DE particles in this mixture were found to have a median aerodynamic diameter of 400 nm. In this study fetal mRNA expression was unaltered, but the placenta exhibited significantly altered levels of cytokine expression**,** as well as histological inflammation and congestion. The most likely interpretation of these findings was that these 400 nm particles did not cross the placental barrier [117], although this was not directly tested. Sugamata *et al.* demonstrated that *in utero* exposure to exhaust-derived nanoparticles resulted in dose-dependent cellular atrophy, apoptosis and damage in the fetal brain [118]. They also visualized what appeared to be DE nanoparticles in cytoplasmic granules inside the offsprings' brains, consistent with passage of these nanoparticles from maternal to fetal tissues, although this was not demonstrated conclusively [118].

In a more recent study, titanium dioxide particles (25-70 nm) were administrated subcutaneously to pregnant mice at 3, 7, 10 and 14 days post-coitum to assess their ability to reach the fetus and affect fetal development [119]. The nanoparticles were subsequently identified in the testes and brains of offspring 6 weeks after birth, and various functional and pathological disorders were detected. The authors hypothesize that the nanoparticles reached the fetal brain because they were administered before the blood-brain barrier had not fully developed [119]. Support for these findings comes from another study that exposed pregnant mice to micron-sized titanium dioxide nanoparticles [120]. An analysis of gene expression in exposed offspring indicated that expression levels of genes associated with apoptosis were altered in the brain of newborn pups, while genes associated with brain development and oxidative stress were altered postnatally. Changes of the expression of genes associated with neurotransmitters and psychiatric diseases were also found. In another study using pregnant mice, the effects of fetal exposure to carbon nanoparticles on the reproductive function in male offspring was investigated [63]. Carbon nanoparticles (14 nm) were instilled intratracheally in the pregnant mice at days 7 and 14 of gestation. A histological examination of gonads from the offspring showed partial vacuolation of seminiferous tubules, plus daily sperm production was significantly decreased. However, carbon nanoparticle administration had no marked effect on body weight, testicle weight, epididymis weight, or serum testosterone concentration. Finally, a toxicology study was carried out on pregnant mice to investigate the effect of carbon nanoparticles (C60 fullerenes) on fetal growth and viability. This molecule was made more hydrophilic with the addition of poly(vinylpyrrolidone) (PVP) and the aqueous suspension was injected intraperitoneally to pregnant mice at varying concentrations. Embryos were removed and examined 18 hours later. Consistent with the studies mentioned above, the fullerine nanoparticles were identified in the embryos and at high doses resulted in significant toxicity, including death [121]. Collectively, these studies strongly suggest that in rodents, the placenta is permeable to some forms of nanoparticles and that these are able to cause significant developmental perturbations and/or toxicity if administered in sufficient quantities in pregnancy.

Quantum dots (Qdots) are small [1-10 nm], highly fluoresecent nanoparticles comprised of a cadmium-containing core enclosed within a functionalised outer shell. In a recently published study, Qdots approximately 3 nm in diameter coated with 3-mercaptopropionic acid [MPA] were administered to pregnant mice to determine their biodistribution [122]. The fetuses were removed at 5.5, 21, and 50 h after injection. The concentration of cadmium atoms in fetal tissue was used as a marker of Qdot distribution in this study to determine the effect of size and surface composition on transplacental transfer. The proportion of cadmium that was transferred to the fetus ranged from 0.23-0.61%. At the higher doses, high rates of fetal demise were observed. The same experiment was repeated with Qdots coated with inorganic silica [SiO_2] or PEG which had a larger diameter of ~4 nm. Again, cadmium was detected in the fetus although less than a third of the levels detected with the MPA coating [122]. This study suggested that quantum dots may be transferred from female mice to their fetuses across the placental barrier. The Qdots may have been modified (degraded) by the placenta during passage. The authors speculated that the Qdots were transferred across the placenta either through transtrophoblastic channels or endocytosis, before diffusing through interstitium and fetal endothelium of placental villi. However, a problem with the study was that flourescence imaging, either *in vivo* or after cryosectioning, failed to identify intact Qdots in fetal tissues. Hence, the cadmium detected may have leached from the Qdots, although the levels of cadmium detected make this an unlikely possibility [122]. In a similar study, monodisperse gold nanoparticles (1.4 nm and 18 nm diameter) were administrated to pregnant rats by tail vein injection**,** and the placental uptake and fetal accumulation assessed [123]. Twenty-four hours after IV-injection**,** low but detectable levels of gold were detected in both the fetus and placenta, with quantities inversely proportional to particle size. The placenta accumulated about 0.03% and 0.0002% of the 1.4 and 18 nm nanoparticle doses, respectively, while fetal tissues retained much lower levels, 0.0006 and 0.00005%, respectively. The possibility that transfer took place *via* the yolk sac was not considered. Although these levels were very low, the fact that even trace amounts of the gold nanoparticles could be detected suggests that, in rodents, the placenta represents only a partial barrier to nanoparticles <20 nm diameter.

Data from Human Studies

There are currently only two papers that have specifically tested nanoparticle transport through the human placenta; both have used the *ex vivo* dually perfused placental cotyledon model to study maternal-to-fetal transfer. In both of these studies [124, 125], the viability of the placental tissue was not affected within the time-frame of the experiments. In the first study, PEGylated gold nanoparticles 10-30 nm diameter were applied to the maternal face of the cotyledon**,** and transfer in the fetal direction was assessed using ICP-MS over 6 h [124]. The authors, Myllynen and colleagues, reported that gold nanoparticles did not cross the perfused human placenta in detectable amounts within this timeframe. However, the nanoparticles were readily taken up by the syncytiotrophoblast cell layer, and concentrations in the maternal perfusate fell by a significant margin, consistent with the placenta acting as a sequestration organ. The authors assumed that the PEGylated nanoparticles entered the syncytium *via* non-specific endocytosis, although this hypothe-

sis was not tested. In another study, using the same placental perfusion method, fluorescently labeled polystyrene beads (50, 80, 240 and 500 nm) were again applied in the maternal to fetal direction over a 6 h period [125]. Particles <240 nm diameter showed significant ability to cross the placental barrier into the fetal circuit, although the transfer was size-dependent. Significant occlusion occurred in particles greater than 80 nm, while the 500 nm particles showed minimal transport. The transport process appeared to be saturable, supporting the conclusion that it involved some form of endocytotic mechanism as opposed to simple diffusion (a receptor-mediated mechanism appears very unlikely). The contrasting results between the two studies are not easy to explain. In both studies**,** significant uptake into the placenta was reported. PEGylated gold nanoparticles [124] with their hydrophilic corona would be expected to have a relatively long half life in the circulation, low toxicity, low immunogenicity [10] and modest ability for cellular uptake depending on the membrane characteristics of the tissue barrier. The polystyrene nanoparticles used by Wick *et al.* [125] were coated with bovine serum albumin which may have fostered some form of endocytosis, although it should be pointed out that albumin is not specifically transported across the human placenta [116]. It would appear that the gold nanoparticles were retained by the basement membrane and prevented from entering the fetal capillaries, whereas the polystyrene nanoparticles were not, for reasons that are not apparent. Importantly, both nanoparticles were able to enter the placenta, suggesting that non-selective uptake mechanisms exist that facilitate nanoparticle entry into the syncytial membrane.

In a series of interesting but as-yet unpublished studies, researchers at the University of Modena and Reggio Emilia have been studying the impact of nanopollution on fetal malformations [126]. They have found evidence that inorganic non-biodegradable nanoparticles, composed of iron, bismuth, titanium and aluminium plus others, are present in various organs from miscarried fetuses. While their findings do not confirm a cause-and-effect association between nanoparticles and birth defects, they do nevertheless suggest that metallic nanoparticulates are getting access to fetal tissues, which is alarming and a cause for concern, although of questionable relevance to biomedical nanoparticles. Somewhat tangential to this discussion, is an interesting study on the passage of antibiotic-loaded dendrimers across intact fetal membranes [127]. The authors of this study used non-laboured intact fetal membranes collected at term to evaluate the use of dendrimer nanoparticles for the intravaginal topical administration of antimicrobial agents. The findings suggest that the transport of dendrimer nanoparticles across fetal membranes would be severely limited when administered as a topical intravaginal formulation [127], confirming expectations based on many other studies [128] that the extraplacental membranes constitute a relatively impermeable barrier to macromolecules.

The extrapolation of data from all these studies must be accompanied by caution due to the variability of nanoparticle composition and surface characteristics and the impact this will have on transfer across the placenta. A great deal of study is required to define the properties of nanoparticles required to determine the extent of placental passage and syncytial uptake [59]. The *ex vivo* dually perfused human placental cotyledon model is the best platform to test the placental perfusion of other nanoparticles, specifically nanoparticle drug delivery systems [59]. Although it is limited in terms of the duration of perfusion that is possible, rendering it unsuitable for the study of long-term/chronic exposures. Finally, it is important to note that even if nanoparticle delivery agents could be developed that completely avoid transplacental transport, this does not necessarily guarantee a lack of toxicity to the fetus. Placental damage as a result of exposure to toxic nanoparticles or their aggregates would have the potential of having indirect adverse effects on fetal growth and development. Furthermore, it was recently demonstrated that nanoparticles can cause toxicity on the opposite side of a cellular barrier without actually crossing the barrier. The fact that this study was performed on transformed placental trophoblast [129] makes the findings more pertinent to placental drug disposition in pregnancy. Note, however, that this study used particularly high doses of toxic heavy metal nanoparticles [129]. The implications for the safety of *in-vivo* drug delivery remain to be determined.

POTENTIAL APPLICATIONS AND BENEFITS OF NANOPARTICLE DRUG DELIVERY IN PREGNANCY

The administration of drugs in pregnancy is surprisingly common, particularly in the first trimester at the time of maximal fetal susceptibility to teratogenesis [73]. A large multi-centre study of drug administration in the USA concluded that almost two-thirds of pregnant women were prescribed some form of pharmaceutical agent prior to delivery. Nearly 1 in 20 of these took a category D or X medication (high risk to the fetus or contraindicated in pregnancy, respectively) after the initial prenatal care visit [130]. Almost one half had taken a category C, D or X medication before delivery. This supports concerns raised by a prior Scandinavian study [131]. A system of drug delivery that could be used to effectively treat maternal conditions without risk to the fetus would be enormously useful. A nanoparticle-based drug delivery system that restricts therapy to maternal tissues but prevents placental passage would accomplish this objective. In any situation involving a pregnant woman in need of pharmacological treatment, fetotoxicity and teratogenicity has a major impact on clinical decision making. Ultimately**,** the decision relies on a risk/benefit assessment for both fetus and mother. Nanoparticle drug delivery systems offer hope for medications that can be specifically designed to avoid placental passage and fetal exposure, dramatically reducing the risk of fetotoxicity and teratogenesis.

There are several examples that illustrate this principle. Antiretroviral drugs directed against the human immunodeficiency virus (HIV) delivered *via* a nanoparticle capsule have been shown to be effective in treating the disease [132]. Such an approach would be useful in treating maternal HIV and preventing vertical transmission. Many nanoparticulate-based drug delivery systems have been investigated for the treatment of cancers. Clearly, pregnant women with malignancies need treatment during pregnancy, but the risks to the fetus of administering chemotherapeutic agents are considerable and present the managing clinician with a difficult di-

lemma. Nanoparticle delivery systems could provide a "fetal-friendly" alternative formulation that can deliver even cytotoxic drugs to maternal tissues without fear of teratogenesis or fetotoxicity. On the other hand, there are circumstances where nanoparticle-based drugs might not be particularly useful in pregnancy. For example, the administration of anticonvulsants for treatment of maternal epilepsy has been associated with both major congenital anomalies and impaired neurocognitive development [133, 134]. However, antiepileptics must cross the blood-brain barrier in order to act. Nanoparticles that are designed to be excluded by the human placenta are also likely to be restricted by the blood-brain barrier [135, 136], unless an endogenous brain-specific endocytotic mechanism can be employed.

There are a number of serious and common conditions of pregnancy where the placenta plays a key pathophysiological role and, as such, could be the target of pharmacological treatment. Here, placental uptake of the drug would be required for efficacy, but passage would need to be prevented for safety, a set of requirements amenable to nanoparticle-based delivery. Relatively large nanoparticles (>100 nm) could be employed to reduce endothelial uptake and target the placenta, as maternal blood has direct access to the syncytial membrane. A clinically relevant example would be preterm birth, a common condition where anti-inflammatory agents might be useful in preventing inflammatory mediator-driven labour and delivery, but could also have serious fetal side effects [135]. Nanoparticles could deliver the anti-inflammatory agent to maternal-facing placental tissues, the site of initiation of inflammation, sparing the fetus from exposure. Alternatively, nanoparticle-delivered gene therapy could be employed to treat some forms of placental dysfunction, a common cause of intrauterine growth restriction, accomplishing efficient trophoblast uptake without placental passage.

In a final scenario, the fetus itself could be a recipient of nanoparticle-targeted drugs. There are receptor-mediated uptake systems in the placenta (e.g. FcR) that could be used by ligand-modified nanoparticles to efficiently deliver drugs to the fetal circulation. Gene therapy strategies to correct serious genetic abnormalities *in utero* could take advantage of this approach, as many nanoparticle formulations have been developed and tested for delivery of DNA-based therapeutics [137-139]. The development of such systems holds great potential promise for maternal-fetal medicine, but would appear to be some way off in light of the lack of robust data on the toxicity, safety and biodistribution of nanoparticles in human pregnancy. A significant amount of work will be required to define the properties of nanoparticles needed to either allow or prevent placental uptake and transfer, together with rigorous nanotoxicology studies, before the concept of "fetal-friendly" nanoparticle-based drug delivery can become a clinical reality.

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